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IN THE PCT UNITED STATES RECEIVING OFFICE

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Applicant: Alan Ebringer  
 International Application No. PCT/GB99/03936  
 International Filing Date: 25 November 1999  
 International Priority Date: 26 November 1998

Title: **DIAGNOSIS OF DEMYELINATING OR SPONGIFORM DISEASE**

Commissioner for Patents  
 BOX PCT  
 Washington, D.C. 20231

**TRANSMITTAL LETTER TO THE UNITED STATES DESIGNED/ELECTED OFFICE (DO/EO/US)  
 CONCERNING A FILING UNDER 35 U.S.C. 371**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31)
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c)(2))
  - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
  - b. ☐ has been communicated by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c) (2)).
  - a. ☐ is attached hereto
  - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☒ are attached hereto (required only if not communicated by the International Bureau). **THE SPECIFICATION AND CLAIMS WERE AMENDED DURING THE INTERNATIONAL EXAMINATION. THE AMENDMENTS ARE ATTACHED TO THE IPER. THE CLAIMS ARE FURTHER HEREBY AMENDED TO DELETE MULTIPLE DEPENDENCIES, AS SHOWN IN THE ATTACHED CLAIMS.**
  - b. ☐ have been communicated by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) - **WILL FOLLOW**
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11 to 20 below concern document(s) or information included:**

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and

3.31 is included.

13. ☐ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information: **COPY OF INTERNATIONAL SEARCH REPORT (PCT/ISA/210); COPY OF INTERNATIONAL APPLICATION WO 00/31545 PUBLISHED UNDER THE PATENT COOPERATION TREATY; COPY OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT (PCT/IPEA/409); RETURN POSTCARD**
21. ☒ The following fees are submitted:

**BASIC NATIONAL FEE (37 CFR 1.492(1) (1) -(5)):**

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO  
 and international Search Report not prepared by the EPO or JPO ..... \$1000.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... \$ 860.00

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International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... \$ 690.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... \$ 100.00

<b>ENTER APPROPRIATE FEE AMOUNT =</b>				<b>\$ 860.00</b>
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$
<b>CLAIMS</b>	<b>NO. FILED</b>	<b>NO. EXTRA</b>	<b>RATE</b>	
Total claims	10	-20 =	x \$18.00	\$
Independent claims	2	-3 =	x \$80.00	\$
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$
<b>TOTAL OF ABOVE CALCULATIONS =</b>				<b>\$ 860.00</b>
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				- \$430.00
<b>SUBTOTAL =</b>				<b>\$ 430.00</b>
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<b>TOTAL NATIONAL FEE =</b>				<b>\$ 430.00</b>
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$
<b>TOTAL FEES ENCLOSED =</b>				<b>\$ 430.00</b>
				Amount to be refunded: \$
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JG08 Rec'd PCT/PTO 16 MAY 2001

- a. ☒ A check in the amount of \$430 to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account No. 03-3975 in the amount of \$ \_\_\_\_\_ to cover the above fees. A duplicate copy of this sheet is enclosed.

The Commissioner is authorized to charge any required additional fees or credit any overpayment to Deposit Account No. 03-3975.

Date: 5-16-01

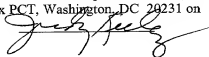
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I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail No. EL 619 083 065 US in an envelope addressed to: Commissioner for Patents, Box PCT, Washington, DC 20231 on May 16, 2001 by Judy Keeley.



DIAGNOSIS OF DEMYELINATING OR SPONGIFORM DISEASE

This invention relates to the diagnosis of demyelinating diseases and spongiform encephalopathies in animals and humans, especially BSE and similar or related diseases in humans.

In our co-pending International application WO97/02667 we have disclosed a new diagnostic test for spongiform encephalopathy and other demyelinating conditions in mammals. The test disclosed in our prior application is based on a model of the genesis of this pathological state which is applicable to the various forms in which it is manifest in humans and other animals. In relation to the bovine spongiform disease this model provides an alternative to the current theory based on the formation of prions. Briefly, the new model is based on the phenomenon of molecular mimicry according to which mammals exposed to certain bacteria having peptide sequences which mimic myelin peptides experience an auto-immune reaction. Foremost among the bacteria that are involved in the induction of the auto-immune reaction are Acinetobacter species, especially Acinetobacter calcoaceticus. The diagnostic test based on the new model opens up the possibility of early treatment of these infections e.g. by use of an appropriate antibiotic to prevent further auto-immune attack on the animal's own myelin.

In our International application WO99/47932, we have confirmed the presence of elevated levels of Acinetobacter IgA antibodies in sera of patients suffering from multiple sclerosis (MS) and Creutzfeld-Jacob disease CJD.

In our priority UK application 9825948.4 we described further tests which confirmed the presence of antibodies to bovine myelin and also to bovine neurofilaments in the sera of cows that have died from BSE. These antibodies are of the IgA type. Similar results have also been obtained with sera from patients suffering from MS and CJD. These findings confirm the validity of the model described above and permit the conclusion that we have discovered a general pattern of the origin of similar diseases that occur or may

occur in vertebrates including humans and other farm animals e.g. in poultry farms. Our latest results also provide the basis of a further test for the early identification of these diseases, especially incipient BSE in cows. This further test may either be alternative to or additional to that based on the detection of IgA antibodies to *Acinetobacter* species e.g. *Acinetobacter calcoaceticus*.

The present invention therefore comprises a method for diagnosing spongiform disease or demyelinating disease in vertebrates, including BSE, MS and CJD, which comprises assaying a biological sample for antibodies, especially IgA antibodies, which bind to myelin and/or neurofilaments or antigenic (immunogenic) parts thereof, including peptide components as hereinafter specified.

The method preferably comprises assaying for antibodies to myelin and /or neurofilaments of vertebrate species e.g. bovine or human species. However, myelin and neurofilaments from other species which are sufficiently homologous to those of bovine or human species to bind to the antibodies under estimation may alternatively be used.

In carrying out the method a positive result is indicated by levels of antibodies at least about two standard deviations above that of control samples.

The invention also comprises a diagnostic kit for the detection of spongiform disease or demyelinating disease in vertebrates comprising, as test antigen, myelin or neurofilaments or antigenic (immunogenic) parts thereof.

The test antigen used in the above defined method and diagnostic kit may be a peptide component of the myelin or neurofilaments, such as one of the following peptides having Sequence ID Nos 1-8, namely,

1. NEALEK      2. LKKVHEE    3. EALEKQL    4. ELEDKQN
5. 2. EALEKQL    6. KKVHEE    7. EIRDRLR    8. EQEIRDRLR

The above sequences have been retrieved from the Protein Information Resource database release 44.

In view of the greater specificity of the IgA antibodies in the immune response it may be concluded that the mechanism of infection with *Acinetobacter* is via the mucous membranes of the body, the primary sites being the gut or the nasal passages. It is possible that the nasal passages are the site of infection, resulting from inhalation of dust formed from dried sewage or animal excrement and carrying *Acinetobacter*. The knowledge of this mechanism implies the need for improved hygiene practices in the rearing of farm animals.

#### Experimental

Assays for the above mentioned organisms are described in our co-pending applications identified above, the contents of which are hereby incorporated by reference. Similar assay procedures using myelin protein or neurofilaments as test antigens are described below.

#### ELISA TEST:

- (1) Aliquots of 200ul of the antigen suspension A or B were absorbed on 96 well flat bottomed rigid polystyrene microtitre plates overnight at 4 deg. Cent. (Antigen A is bovine myelin from Sigma Chemical Company, Fancy Road, Poole, Dorset, BH12 4XA, UK, at a concentration of 5ug/ml and antigen B is bovine neurofilaments from Sigma also at a concentration of 5ug/ml).
- (2) The plates are then washed 3 times with phosphate buffered saline (PBS) 0.1% (v/v) Tween 20.
- (3) Aliquots of 300ul of blocking solution (0.2% w/v ovalbumin, 0.1% v/v Tween) in PBS is added to each well and incubated for one hour at 37 deg. Cent.
- (4) The plates are then washed 3 times with PBS. Tween 20.

(5) Aliquots of 200ul serum samples (test or control) diluted 1/200 in PBS. Tween is added and incubated for 2 hours at 37 deg. Cent.

(6) The plates are then washed 3 times with PBS. Tween 20.

(7) Aliquots of 200ul of peroxidase conjugated rabbit anti-cow IgA (alpha chain)

5 diluted 1/4000 with PBS. Tween are added and incubated for 2 hours at 37 deg. Cent.

(8) The plates are then washed 3 times with PBS. Tween 20.

(9) The development of the colorimetric assay takes place at room temperature for 20 minutes, after the addition of 200ul per well of 0.5 mg/ml (2,2'-azinobis (3-ethylbenz-thiazoline-6-sulphonic acid) in citrate/ phosphate buffer, pH 4.1, containing 0.98 mM hydrogen peroxide.

10 (10) The reaction is then stopped with 100ul of 2 mg/ml sodium fluoride and optical densities measured at a wavelength of 630 nm with a micro-ELISA plate reader.

(11) All assays are done under coded conditions, in that the tester is unaware of the origin of the serum being studied (Test or control).

15 (12) All tests are done in duplicate.

The foregoing test procedure may be carried out in the same manner using human myelin or neurofilaments or peptides derived therefrom.

20 This assay is a novel way of diagnosing cattle suffering from bovine spongiform encephalopathy and humans suffering from MS and CJD in that it describes a test where antibodies to two brain antigens can be determined in bovine or human sera. Any reading in excess of 2 standard deviations of the healthy controls would indicate a positive response. Furthermore the test should be positive (above 2 standard deviations)

25 for both antigens: (A) Bovine myelin protein and (B) Bovine neurofilaments.

This is the first assay that describes measurements of autoantibodies to brain antigens in BSE affected cattle and patients with MS and CJD.

30 Results for BSE are shown in the accompanying Figures 1 and 2.

Those for MS and CJD are shown in the accompanying Figure 3.

The tests described in our above-mentioned International applications may be combined with that of the present invention. This combined test is particularly suitable for use in testing for BSE. This combined test may be termed the "MAN test" and is based on separate measurements of autoantibodies to bovine myelin (white matter of the brain) and to bovine neurofilaments (gray matter of the brain), as well as to specific antibodies to the saprophytic bacterium *Acinetobacter calcoaceticus*.

The auto-antibodies to bovine myelin and to bovine neurofilaments and antibodies to *Acinetobacter* are measured as previously described, for each animal tested. The MAN index is then obtained by multiplying the optical densities according to the following algorithm: =

Myelin IgA autoantibody x *Acinetobacter* antibody x Neurofilaments autoantibody  
i.e. the multiplication product  $M \times A \times N$ .

The accompanying Figure 4 shows the results of this test when compared to healthy "organic" controls or to controls (CVL) suffering from other diseases. (CVL = Central Veterinary Laboratory, UK, from where these sera from animals with other diseases were obtained).

The MAN test is calibrated against "organic" farm controls, that is animals coming from a farm where the feedstuffs consist of grass and hay only. The MAN test is an empirical test, in that very low values are obtained for the MAN index, when healthy cows only are tested.

A positive response is recognised when the MAN index is 3 standard deviations above the value found in controls, when testing the serum of a cow suspected of having BSE.



CLAIMS

1. A method for diagnosing spongiform disease or demyelinating disease in vertebrates, including BSE, MS and CJD, which comprises assaying a biological sample for antibodies which bind to myelin and/or neurofilaments or to one or more antigenic (immunogenic) parts thereof.

2. A method according to claim 1, in which the antibodies are IgA antibodies.

3. A method according to claim 1, in which the assay is for antibodies that bind to vertebrate myelin and/or neurofilaments or parts thereof.

4. A method according to claim 3, in which the vertebrate is bovine or human.

5. A method according to claim 4, in which the test antigen is a peptide selected from the group consisting of peptides having sequences identified as Sequence ID Nos. 1 to 8 hereinbefore specified.

6. A method according to any of claims 1, in which a positive result is indicated by levels of antibodies at least about two standard deviations above that of control samples.

7. A method according to any of the preceding claims combined with an assay for antibodies to Acinetobacter species.

8. A diagnostic kit for the detection of spongiform disease or demyelinating disease in vertebrates comprising, as test antigen, myelin and/or neurofilaments and/or one or more parts thereof.

9. A diagnostic kit according to claim 8, in which the test antigen is a peptide having a sequence selected from the group consisting of Sequence ID Nos. 1 to 8 specified hereinbefore.

- 5 10. A diagnostic kit according to claim 8, containing as test antigens myelin, neurofilaments, and *Acinetobacter calcoaceticus*.

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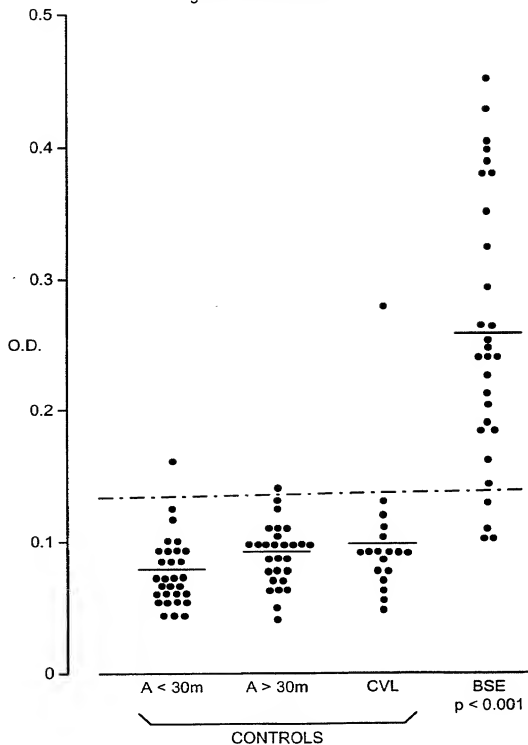
Diagnosis of Demyelinating or Spongiform DiseaseABSTRACT

- 5 A method for diagnosing spongiform disease or demyelinating disease in vertebrates, including BSE, MS and CJD, which comprises assaying a biological sample for antibodies which bind to myelin and/or neurofilaments or to one or more antigenic (immunogenic) parts thereof.

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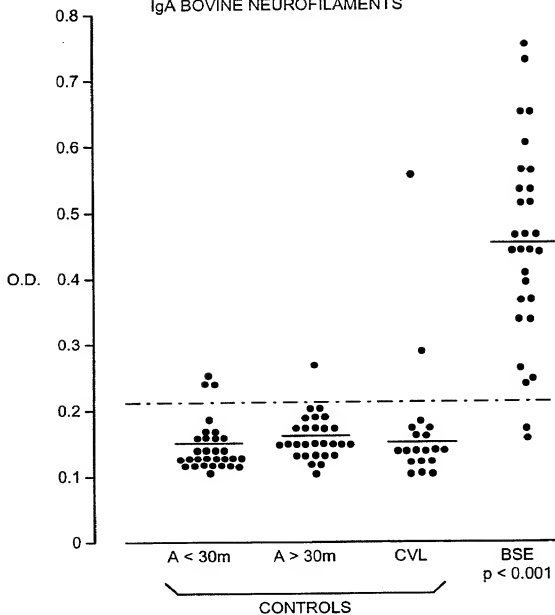
FIG. 1  
IgA BOVINE MYELIN



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FIG. 2

IgA BOVINE NEUROFILAMENTS



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FIG. 3

IgA BOVINE MYELINE

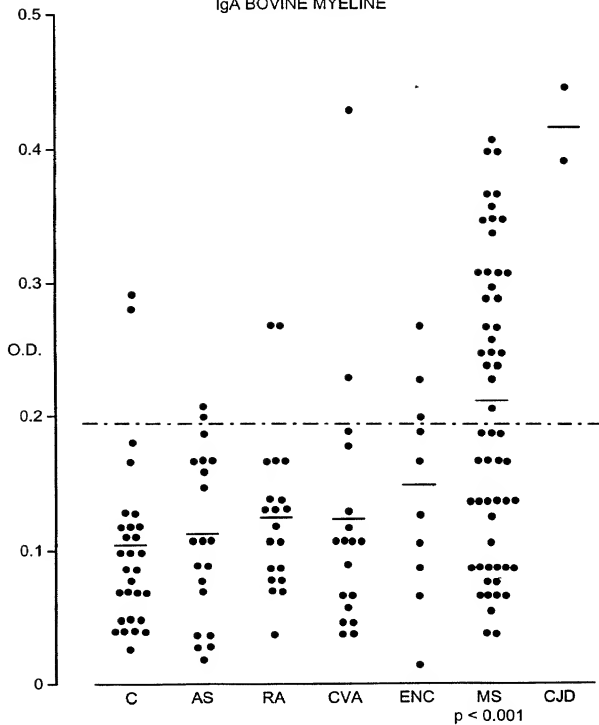
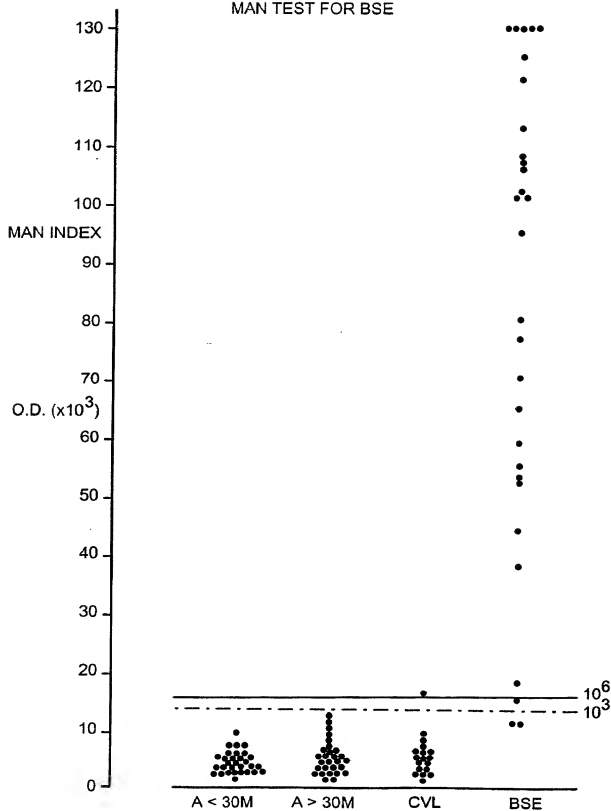


FIG. 4

MAN TEST FOR BSE



## RULE 63 (37 C.F.R. 1.63)

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION  
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled — **DIAGNOSIS OF SPONGIFORM OR DEMYELINATING DISEASE**. The specification was filed in the U.S. Patent Office on May 16, 2001 under Serial No 09/856,086 by way of entry into the national phase of Chapter II of International Application Number PCT/GB99/03936 filed November 25, 1999, which in turn claims priority from British Patent Application Number GB 9825948.4 filed November 26, 1998.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate filed by me or my assignee disclosing the subject matter claimed in this application and having a filing date (1) before that of the application on which priority is claimed, or (2) if no priority claimed, before the filing date of this application:

## PRIOR FOREIGN APPLICATION(S):

Number	Country	Day/Month/Year Filed	Date First Laid Open or Published	Date Patented or Granted	Priority Claimed? Yes/No
PCT/GB99/03936	PCT	25 November 1999	02 June 2000		YES
GB 9825948.4	GB	26 November 1998			YES

I hereby claim domestic priority benefit under 35 U.S.C. 119/120/365 of the indicated United States applications listed below and PCT international applications listed above or below and, if this is a continuation-in-part (CIP) application, insofar as the subject matter disclosed and claimed in this application is in addition to that disclosed in such prior applications, I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56 which became available between the filing date of each such prior application and the national or PCT international filing date of this application:

## PRIOR U.S. PROVISIONAL, NONPROVISIONAL AND/OR PCT APPLICATION(S)

Application No.	Day/Month/Year Filed	Status (Pending, Abandoned, Patented)	Priority Claimed? YES/NO

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are



made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

And I hereby appoint David H. Jaffir, and the firm of Pillsbury Winthrop LLP, 2550 Hanover Street, Palo Alto, California 94304-1115, telephone number (650) 233-4510 (to whom all communications are to be directed), and the below-named persons (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent, and I hereby authorize them to delete persons no longer with their firm and to act and rely on instructions from and communicate directly with the person/assignee who first sent this case to them and by whom I hereby declare that I have consented after full disclosure to be represented unless/until I instruct the above firm and/or a below attorney in writing to the contrary.

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Date

20<sup>th</sup> JULY 2001

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